Intended Use

NOVA Gel™ Sm/RNP is a double diffusion test system for the titering and semi-quantitative determination of autoantibodies to Sm/RNP, an extractable nuclear antigen (ENA), in human serum. The presence of antibodies to Sm/RNP can be used in conjunction with other serological tests and clinical findings to aid in the diagnosis of Systemic Lupus Erythematosus (SLE) and other related connective tissue diseases.

Summary and Explanation of the test

The term "Anti-nuclear antibodies" (ANA) describes a variety of autoantibodies that react with constituents of cell nuclei including DNA, RNA and several proteins and ribonucleoproteins. These autoantibodies occur with high frequency in patients with connective tissue or rheumatic diseases, especially SLE. Virtually all SLE patients are ANA positive. This diagnostic sensitivity has led to the incorporation of ANA testing into the 1982 Revised Criteria for the Classification of Systemic Lupus Erythematosus by an Arthritis and Rheumatism Association subcommittee. While ANA testing is an excellent screening test for SLE (a negative result virtually rules out active SLE) it is by no means a specific test.

The current trend in diagnosing and managing connective tissue disease is to combine a sensitive ANA screening test with more specific follow up tests such as dsDNA and/or ENA. The ENA tests are especially important as they provide both diagnostic and prognostic information.

Autoantibodies to the Sm antigen are highly specific for SLE and, as with antibodies to dsDNA, are considered a marker antibody. Antibodies to Sm have been included in the Arthritis and Rheumatism subcommittee criteria for the classification of SLE. Autoantibodies to RNP are found in patients with SLE and also in some other connective tissue diseases. Autoantibodies to RNP are associated with a relatively benign disease course with lower incidence of renal disease, while patients with Sm antibody have a higher incidence of renal disease and central nervous system involvement.

Principles of the Procedure

The double diffusion test, described by Ouchterlony, allows an antigen solution to passively diffuse through a gel support matrix towards a patient sample and/or antibody containing control. Specially shaped wells punched into the gel matrix serve as reservoirs from which the antigen and antibody solutions diffuse towards each other. At the point of antigen-antibody equivalence, a visible precipitin line forms in the gel. The immunologic status of a patient can then be determined by observing the patterns of adjacent precipitin lines formed in the gel by a control sample of known specificity and an unknown patient serum.

The double diffusion technique can be used with a purified Sm/RNP preparation, a known Sm or RNP autoantibody control sample and a patient serum suspected to contain Sm and/or RNP autoantibodies to show immunologic identity, non-identity or partial identity. Confirmation of Sm and/or RNP autoantibody activity in the patient’s serum can be made if its precipitin line characteristically merges with the line formed by a known control sample.

In certain instances, a semi-quantitative determination of the amount of Sm and/or RNP autoantibody in a patient’s serum is desired. In these cases the NOVA Gel “T” plate can be utilized to assay a serially diluted patient sample. The reciprocal of the highest dilution which forms a precipitin line can then be reported as the titer of the Sm and/or RNP autoantibody for the patient. Correct double diffusion testing procedure calls for the assay of multiple antigen-antibody concentration ratios in order to prevent antigen or antibody excess. Incorrect or misleading precipitin
line formation can occur from antigen or antibody excess. To prevent this, dilutions of the patient serum should be assayed against the same antigen preparation. This is concurrently accomplished while titering the patient’s sample on the NOVA Gel “T” plate.

**Reagents**

1. Nova Gel “T” screening gel plates with 7 wells each, containing stabilizers and 0.09% sodium azide
2. 0.4mL Sm/RNP antigen (from calf thymus), lyophilized in buffer containing stabilizers and 0.09% sodium azide
3. 0.7mL Sm Control, human serum containing autoantibodies to Sm in buffer containing 0.09% sodium azide
4. 0.7mL RNP Control, human serum containing autoantibodies to RNP in buffer containing 0.09% sodium azide

**Warnings**

1. All human source material used in the preparation of controls for this product has been tested and found negative for antibody to HIV, HbsAg, and HCV by FDA cleared methods. No test method, however, can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Sm Control and RNP Control should be handled in the same manner as potentially infectious material.9
2. Sodium Azide is used as a preservative. Sodium Azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
3. Use appropriate personal protective equipment while working with the reagents provided.
4. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

**Precautions**

1. This product is for *In Vitro* Diagnostic Use.
2. Substitution of components other than those provided in this system may lead to inconsistent results.
3. It is recommended that *sterile distilled* or *deionized* water be used to rehydrate the antigen.
4. A variety of factors influence the assay performance. These include the starting temperature of the reagents, the prevention of reagents or patient samples overflowing the wells and the quality of the water used to rehydrate the antigen. Careful attention to consistency is required to obtain accurate and reproducible results.
5. Strict adherence to the protocol is recommended.

**Storage Conditions**

Store all the kit reagents at 2-8°C. **Do not freeze.** Reagents are stable until the expiration date when stored and handled as directed. Once rehydrated, the antigen is stable for 2 weeks at 2-8°C. If desired, 100μL aliquots of antigen can be prepared and stored at ≤ -70°C. Antigen stored in this manner is stable for at least 1 year.

**Specimen Collection**

This procedure should be performed with a serum specimen. Addition of azide or other preservatives to the test samples may adversely affect the results. Microbially contaminated, heat-treated, or specimens containing visible particulates should not be used. Grossly hemolyzed or lipemic serum specimens should be avoided.
Following collection, the serum should be separated from the clot. NCCLS Document H18-A2 recommends the following storage conditions for samples: 1) Store samples at room temperature no longer than 8 hours. 2) If the assay will not be completed within 8 hours, refrigerate the sample at 2-8°C. 3) If the assay will not be completed within 48 hrs, or for shipment of the sample, freeze at -20°C or lower. Frozen specimens must be mixed well after thawing and prior to testing.

**Procedure**

**Materials provided**
- 8 prepunched NOVA Gel titering "T" plates
- 2 0.4mL Sm/RNP antigen, lyophilized
- 1 0.7mL Sm Control
- 1 0.7mL RNP Control

**Additional Materials Required But Not Provided**
- Micropipets to deliver 15-100μL volume
- Disposable micropipet tips
- 0.85% Saline
- Distilled or deionized water (preferably sterile)
- Light source or gel viewing box

**Method**

1. **Rehydrate the Sm/RNP Antigen**
   Carefully open an antigen vial and add 0.4mL of distilled or deionized water to the lyophilized antigen. Sterile distilled or deionized water is highly recommended for optimal product performance. Allow the rehydrated antigen to stand for 5 minutes, then swirl to mix thoroughly before use.

2. **Filling the Gel Plate**
   Prepare a serial twofold dilution of the patient sample in 0.85% saline. Load the gel plate wells with antigen (approximately 80μL), control (approximately 80μL), and patient sample dilutions (approximately 80μL) as illustrated. Exact fill amounts are not critical; however DO NOT OVERFLOW THE WELLS. The control may be filled directly from plastic squeeze bottles or micropipets. If using squeeze bottles, place the dispenser tip into the well and gently squeeze until the control level nears the top of the well. Cover the plate immediately after filling.

3. **Incubation**
   Allow the covered plate(s) to incubate at room temperature on a stable, flat surface for 24 hours. Examine the plate(s) and note any precipitin lines that form between the patient sample and the antigen. Examine the relationship of this line to either control’s precipitin line. Reexamine the plate(s) again at 48 hours to check for any newly developed lines prior to final interpretation of the results.
Quality Control
Sm and/or RNP Controls should be run on every plate to insure that all reagents and procedures have performed properly. Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing at \(-70^\circ\text{C}\). In order for the test results to be considered valid, a precipitin line must be visible between the Sm and/or RNP Controls and the Sm/RNP antigen wells.

Interpretation of Results
For optimal observation of the precipitation lines, the following procedure is recommended:
1. Remove the plate lid.
2. Hold the plate in a position which minimizes the gel surface reflection.
3. Illuminate the gel plate from the side (i.e. parallel to the surface of the gel). Note: Be sure the light source is shielded from direct view.
4. Precipitin lines are best seen when contrasted against a dark background and viewed from an angle.
5. A magnifier may aid in the observation of faint lines or for distinguishing non-identity samples.

Negative Reaction. A sample is considered negative if no visible precipitin line forms between the patient wells and the antigen well after 48 hours.

Specificity Interpretation. In double diffusion, specificity is determined by noting the relationship of the sample precipitin lines with the known control lines. The following possibilities can occur with the NOVA Gel\textsuperscript{TM} Sm/RNP test:

Fig. 1: Non-Identity

The patient precipitin line crosses or shows **non-identity** with both the Sm and the RNP controls. This indicates that the patient reacts with an ENA antigen other than Sm or RNP.

Fig. 2: Sm Identity

The patient precipitin line merges or shows **identity** with the Sm control line. In this case the patient has autoantibodies to Sm. The titer of the sample is equivalent to the reciprocal of the highest dilution which forms a precipitin line with the antigen. In this case the patient can be said to have antibody to Sm and a titer of 8.
The patient precipitin line merges or shows **identity** with the RNP control line. In this case the patient can be said to have antibody to RNP and a titer of 4. Note the spur with the Sm control. This is non-identity for Sm. It shows partial identity with the RNP portion of the Sm control.

**Limitations of the Procedure**

1. Although unlikely with this test because of the serial dilution of the sample, prozoning is a possibility. Prozone occurs when an overwhelming amount of patient autoantibody is present relative to the antigen (antibody excess), causing the precipitin line to form at the antigen well face. In these cases retesting of possible positive samples at higher dilutions in saline is recommended to prevent a false negative test interpretation.
2. If non-sterile or contaminated water is used to rehydrate the antigen, the antigen may suffer immediate or progressive degradation. In this case a very light precipitin line or no line may result.
3. The Sm Control is not monospecific and may contain some RNP. The Sm Control will show partial identity with a RNP patient or control.
4. It may be possible to observe a spur that points toward the RNP control well with a serum that shows identity with the Sm control. This may indicate the presence of RNP antibodies in the anti-Sm patient serum.
5. Results of this assay should be used in conjunction with clinical findings and other serological tests.
6. The assay performance characteristics have not been established for matrices other than serum.

**Expected Values**

NOVA Gel™ Sm/RNP double diffusion test was used to evaluate a variety of connective tissue disease patients as well as 200 random blood donors. The results appear below:

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Number</th>
<th>Number Positive Sm</th>
<th>NOVA Gel™ ENA Test Sm Positive</th>
<th>NOVA Gel™ ENA Test RNP Positive</th>
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<tbody>
<tr>
<td>SLE</td>
<td>105</td>
<td>15</td>
<td>20</td>
<td></td>
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<tr>
<td>Drug Induced Lupus</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td></td>
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<tr>
<td>Rheumatoid Arthritis</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td></td>
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<td>Scleroderma</td>
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<td>0</td>
<td>1</td>
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<td>Dermatomyositis</td>
<td>14</td>
<td>0</td>
<td>1</td>
<td></td>
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<tr>
<td>Sjogren's Syndrome</td>
<td>14</td>
<td>0</td>
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<td>Normals</td>
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References